Generating gene knockout rats by homologous recombination in embryonic stem cells.

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Public Summary:
We describe here a detailed protocol for generating gene knockout rats by homologous recombination in embryonic stem (ES) cells. This protocol comprises the following procedures: derivation and expansion of rat ES cells, construction of gene-targeting vectors, generation of gene-targeted rat ES cells and, finally, production of gene-targeted rats. The major differences between this protocol and the classical mouse gene-targeting protocol include ES cell culture methods, drug selection scheme, colony picking and screening strategies. This ES cell-based gene-targeting technique allows sophisticated genetic modifications to be performed in the rat, as many laboratories have been doing in the mouse for the past two decades. Recently we used this protocol to generate Tp53 (also known as p53) gene knockout rats. The entire process requires ~1 year to complete, from derivation of ES cells to generation of knockout rats.

Scientific Abstract:
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